FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)
213993US0PCT	09/926169

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR00/00658	17 March 2000	19 March 1999

TITLE OF INVENTION
SCREENING METHOD INVOLVING MGDG SYNTHASE

APPLICANT(S) FOR DO/EO/US
MARECHAL Eric et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☒ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Request for Consideration of Documents Cited in International Search Report/Notice of Priority
PCT/IB/304/Amended Sheets (Pages 22 and 23)/Drawings (5 sheets)
PCT/IB/308/Sequence Listing (2 pages)

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/926169	PCT/FR00/00658	213993US0PCT

24. The following fees are submitted:	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**		
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$860.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$130.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	- 20 =	0	x $18.00	**$0.00**	
Independent claims	- 3 =	0	x $80.00	**$0.00**	
Multiple Dependent Claims **(check if applicable).**			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$990.00**	
☐ Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$0.00**	
SUBTOTAL =				**$990.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$990.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**	
TOTAL FEES ENCLOSED =				**$990.00**	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of **$990.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **15-0030** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

22850

Surinder Sachar
Registration No. 34,423

Surinder Sachar
SIGNATURE

Norman F. Oblon
NAME

24,618
REGISTRATION NUMBER

Sept. 18 2001
DATE

#4

Docket No.: 213993US0 PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :

Eric MARECHAL, et al. : ATTN: APPLICATION DIVISION

SERIAL NO: 09/926,169 :

FILED: SEPTEMBER 18, 2001 :

FOR: SCREENING METHOD INVOLVING MGDG SYNTHASE

PRELIMINARY AMENDMENT AND STATEMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows.

IN THE SPECIFICATION

Please replace the paragraph starting on page 6, line 28 with the following:

- figure 2 is a comparison of spinach, cucumber and Arabidopsis MGDG synthase; figure 2A corresponds to a comparison of the amino acid sequences deduced from the cDNAs encoding the various MGDG synthases; in this figure, atMGD A (SEQ ID No. 10) and atMGD B (SEQ ID No. 11) correspond to sequences derived from *Arabidopsis thaliana*, csMGD A (SEQ ID No. 9) corresponds to a sequence derived from *Cucumis sativa* and soMGD A (SEQ ID No. 8) corresponds to a sequence derived from *Spinacia oleracea*. * and : represent symbols for the identical amino acids and the conserved substitutions, respectively; h1 to h7 correspond to 7 putative α-helices; figure 2B represents a phylogenic tree of mature MGDG synthases;

IN THE CLAIMS

Please cancel Claims 1-3 and 10-11.

Please amend the claims as shown on the attached marked-up copy to read as follows:

--4. (Amended) A method for screening and for selecting antiparasitic agents, herbicides or combinations thereof, comprising

-incubating a substance to be tested with an MGDG synthase or with a plastidial membrane isolated from a plant, and

-measuring the specific enzymatic activity, after said incubation.

5. (Amended) The method as claimed in claim 4, wherein said MGDG synthase has an initial specific activity of between 0.1 and 120 μmol of galactose incorporated/h/mg of protein.

6. (Amended) The method as claimed in claim 4, wherein the MGDG synthase/substance to be tested incubation is carried out in an incubation medium containing a buffer adjusted to a pH of between 6 and 9, in the presence of detergents, a reducing agent, phosphatidylglycerol, a salt or combinations thereof.

7. (Amended) The method as claimed in claim 6, wherein the incubation medium further comprises 50 mM of MOPS-NaOH, 4.5 mM of CHAPS, 1 mM of DTT, 1.3 mM of phosphatidylglycerol, 250 mM of KH_2PO_4/K_2HPO_4 and 250 mM of KCl, and has a pH of 7.8

8. (Amended) The method according to claim 4, wherein the MGDG synthase is of plant origin and is selected from the group consisting of the purified MGDG synthases A, recombinant MGDG synthases A, purified MGDG synthases B, and recombinant MGDG synthases B.

9. (Amended) The method as claimed in claim 4, wherein said apicomplex parasite is selected from the group consisting of *Plasmodium, Toxoplasma* and *Eimeria*.--

Please add new Claims 12-17 as follows:

--12. (New) A pharmaceutical composition comprising an MGDG synthase inhibitor and a pharmaceutically-acceptable carrier or excipient.

13. (New) A method for treating an animal, including a human, having an apicomplex parasite, comprising administering the pharmaceutical composition claimed in claim 12 to said animal.

14. (New) The method as claimed in Claim 13, wherein the apicomplex parasite is selected from the group consisting of *Plasmodium, Toxoplasma* and *Eimeria.*

15. (New) A pharmaceutical composition comprising an MGDG synthase inhibitor and a pharmaceutically-acceptable carrier or excipient, wherein said MGDG synthase inhibitor is selected by the method claimed in claim 4.

16. (New) An herbicide comprising an MGDG synthase inhibitor and a carrier, wherein said MGDG synthase inhibitor is selected by the method claimed in claim 4.

17. (New) A method comprising treating a plant with the herbicide claimed in claim 16, comprising applying said herbicide to a plant.--

REMARKS

Claims 4-9 and 12-17 are active in the present application. Claims 1-3 and 10-11 have been cancelled. Claims 12-17 are new claims. Support for the new claims is found in the original claims. Claims 4-9 have been amended to remove multiple dependencies and for clarity. By virtue of the present amendment, a fee is not required for multiple dependent claims.

No new matter is added by the present amendment.

Applicants have now submitted an substitute Sequence Listing and a corresponding computer-readable Sequence Listing. The sequence information recorded in the corresponding computer-readable Sequence Listing is identical to the paper copy of the substitute Sequence Listing. Support for all of the sequences listed in the substitute Sequence Listing is found in the present application as originally filed. No new matter is

believed to have been introduced by the submission of the substitute Sequence Listing and the corresponding computer-readable Sequence Listing.

Applicants submit that the present application is ready for examination on the merits. Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

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22850

#4

MARKED-UP COPY

IN THE SPECIFICATION

Please replace the paragraph starting on page 6, line 28 with the following:

- figure 2 is a comparison of spinach, cucumber and Arabidopsis MGDG synthase; figure 2A corresponds to a comparison of the amino acid sequences deduced from the cDNAs encoding the various MGDG synthases; in this figure, atMGD A (SEQ ID No. 10) and atMGD B (SEQ ID No. 11) correspond to sequences derived from *Arabidopsis thaliana*, csMGD A (SEQ ID No. 9) corresponds to a sequence derived from *Cucumis sativa* and soMGD A (SEQ ID No. 8) corresponds to a sequence derived from *Spinacia oleracea*. * and : represent symbols for the identical amino acids and the conserved substitutions, respectively; h1 to h7 correspond to 7 putative α-helices; figure 2B represents a phylogenic tree of mature MGDG synthases;

IN THE CLAIMS

Please amend the claims as follows:

--Claims 1-3 and 10-11 (Cancelled).--

--4. (Amended) A method for screening and for selecting antiparasitic agents, [and/or] herbicides or combinations thereof, [characterized in that it comprises:] comprising,

-incubating a substance to be tested with an MGDG synthase or with a plastidial membrane isolated from a plant, and

-measuring the specific enzymatic activity, after said incubation.

5. (Amended) The method as claimed in claim 4, [characterized in that] wherein said MGDG synthase [preferably] has an initial specific activity of between 0.1 and 120 μmol of galactose incorporated/h/mg of protein.

6. (Amended) The method as claimed in claim 4 [or claim 5 characterized in that], wherein the MGDG synthase/substance to be tested incubation is carried out in an incubation medium containing a buffer adjusted to a pH of between 6 and 9, in the presence of detergents, [of] a reducing agent, [of] phosphatidylglycerol, [and of] a salt or combinations thereof.

7. (Amended) The method as claimed in claim 6, [characterized in that] wherein the incubation medium [preferably contains] further comprises 50 mM of MOPS-NaOH, [pH 7.8,] 4.5 mM of CHAPS, 1 mM of DTT, 1.3 mM of phosphatidylglycerol, 250 mM of KH_2PO_4/K_2HPO_4 and 250 mM of KCl and has a pH of 7.8.

8. (Amended) The method according to [any one of claims 4 to 7, characterized in that] claim 4, wherein the MGDG synthase is of plant origin and is selected from the group consisting of the purified [or recombinant] MGDG synthases A, recombinant MGDG synthases A, purified MGDG synthases B, and recombinant [and] MGDG synthases B.

9. (Amended) The method as claimed in [any one of claims 4 to 8, characterized in that] claim 4, wherein said apicomplex parasite is selected from the group consisting of *Plasmodium, Toxoplasma* and *Eimeria*.--

--Claims 12-15 (New).--

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Docket No. 213993US0PCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: Eric MARECHAL, et al.

SERIAL NUMBER: 09/926,169 ATTN: APPLICATION BRANCH

FILING DATE: September 18, 2001

FOR: SCREENING METHOD INVOLVING MGDG SYNTHASE

FILING OF DECLARATION UNDER 37 CFR 1.53(f)

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Responsive to the notification dated **January 15, 2002**, and in accordance with the provisions of 37 CFR 1.53(f), Applicants submit herewith a Rule 63 Declaration.

The required fee was paid at the time of filing the application.

In light of the foregoing, this application is deemed to be in proper condition for examination and such favorable action is earnestly solicited.

Respectfully Submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

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#4

SEQUENCE LISTING

<110> MARECHAL, ERIC

BLOCK, MARYSE

JOYARD, JACQUES

DOUCE, ROLAND

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Tyr Gly Thr Ser Lys Ser Ala Val Ser Val Thr Ser Gly Asp Gly Glu
            20                  25                  30

Lys Thr His Arg His Thr His His His Ile His Arg Ile Lys Ser Tyr
        35                  40                  45

Asp Asp Ile Asp Glu Asp Glu Ser Ser Leu Glu Leu Ile Gln Ile Gly
    50                  55                  60

Ala Glu Arg Thr Lys Asn Val Leu Ile Leu Met Ser Asp Thr Gly Gly
65                  70                  75                  80

Gly His Arg Ala Ser Ala Glu Ala Ile Arg Asp Ala Phe Lys Ile Glu
                85                  90                  95

Phe Gly Asp Lys Tyr Arg Val Ile Val Lys Asp Val Trp Lys Glu Tyr
            100                 105                 110

Thr Gly Trp Pro Leu Asn Asp Met Glu Arg Ser Tyr Lys Phe Met Val
        115                 120                 125

Lys His Val Gln Leu Trp Lys Val Ala Phe His Ser Thr Ser Pro Lys
    130                 135                 140

Trp Ile His Ser Cys Tyr Leu Ala Ala Ile Ala Ala Tyr Tyr Ala Lys
145                 150                 155                 160
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Glu Val Glu Ala Gly Leu Met Glu Tyr Lys Pro Glu Ile Ile Ile Ser
                165                 170                 175

Val His Pro Leu Met Gln His Ile Pro Leu Trp Val Leu Lys Trp Gln
            180                 185                 190

Glu Leu Gln Lys Arg Val Leu Phe Val Thr Val Ile Thr Asp Leu Asn
        195                 200                 205

Thr Cys His Pro Thr Trp Phe His Pro Gly Val Asn Arg Cys Tyr Cys
    210                 215                 220

Pro Ser Gln Glu Val Ala Lys Arg Ala Leu Phe Asp Gly Leu Asp Glu
225                 230                 235                 240

Ser Gln Val Arg Val Phe Gly Leu Pro Val Arg Pro Ser Phe Ala Arg
                245                 250                 255

Ala Val Leu Val Lys Asp Asp Leu Arg Lys Glu Leu Glu Met Asp Gln
            260                 265                 270

Asp Leu Arg Ala Val Leu Leu Met Gly Gly Gly Glu Gly Met Gly Pro
        275                 280                 285

Val Lys Glu Thr Ala Lys Ala Leu Glu Glu Phe Leu Tyr Asp Lys Glu
    290                 295                 300

Asn Arg Lys Pro Ile Gly Gln Met Val Val Ile Cys Gly Arg Asn Lys
305                 310                 315                 320

Lys Leu Ala Ser Ala Leu Glu Ala Ile Asp Trp Lys Ile Pro Val Lys
                325                 330                 335

Val Arg Gly Phe Glu Thr Gln Met Glu Lys Trp Met Gly Ala Cys Asp
            340                 345                 350

Cys Ile Ile Thr Lys Ala Gly Pro Gly Thr Ile Ala Glu Ser Leu Ile
        355                 360                 365
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Arg Ser Leu Pro Ile Ile Leu Asn Asp Tyr Ile Pro Gly Gln Glu Lys
370 375 380

Gly Asn Val Pro Tyr Val Val Glu Asn Gly Ala Gly Val Phe Thr Arg
385 390 395 400

Ser Pro Lys Glu Thr Ala Arg Ile Val Gly Glu Trp Phe Ser Thr Lys
405 410 415

Thr Asp Glu Leu Glu Gln Thr Ser Asp Asn Ala Arg Lys Leu Ala Gln
420 425 430

Pro Glu Ala Val Phe Asp Ile Val Lys Asp Ile Asp Glu Leu Ser Glu
435 440 445

Gln Arg Gly Pro Leu Ala Ser Val Ser Tyr Asn Leu Thr Ser Ser Phe
450 455 460

Ala Ser Leu Val
465

SCREENING METHOD INVOLVING MGDG SYNTHASE

The present invention relates to a method for screening and for selecting antiparasitic agents (parasites of the apicomplex phylum) and/or herbicides.

Apicomplexes are single-cell parasites responsible for diseases which are among the most serious for the human species: malaria, the primary deadly disease in the world, and toxoplasmosis, one of the two most common opportunist infections in individuals suffering from AIDS. These infectious diseases are spreading, while no treatment at this time makes it possible to eradicate the parasites which cause them: *Plasmodium* which are found in the hepatic cells and red blood cells of individuals suffering from malaria, and *Toxoplasma* which invades, among other things, the brain of individuals suffering from toxoplasmosis.

Specifically, according to the World Health Organization (WHO), malaria affects more than 500 million human beings and causes 2.5 million deaths per year. Malaria kills half the children under the age of 5 in Africa. 40% of the world population live in regions where malaria is present, and these regions spread each year. Pesticide treatments have caused the mosquitoes which are vectors for the parasites (anopheles) to become resistant and the parasite itself (4 species of *Plasmodium*, including *Plasmodium falciparum* for 95% of cases) is becoming increasingly resistant to known treatments (in particular chloroquine derivatives). According to estimates, malaria is the first or second (after diarrhea) deadliest disease in the world. The direct and indirect cost of malaria in Africa has gone from 800 million dollars in 1987 to more than 2 billion dollars in 1998. The resistance to treatments and the spread of the regions where malaria is present make this scourge a major challenge of the 21st century.

According to the *National Institute of Health* (NIH), toxoplasmosis is the primary brain infection in individuals suffering from AIDS. The parasite (*Toxoplasma gondii*) is common and it may be considered that one person in two has been infected, either by eating incorrectly cooked meat or by coming into contact with domestic cats. Toxoplasmosis is serious only in frail individuals, in particular human fetuses and individuals suffering from AIDS. In the case of AIDS, the patients exhibiting a $CD4^+$ level $< 100/mm^3$ develop symptoms of toxoplasmosis, in general by reactivation of a prior infection. The known treatments (sulfadiazine and pyrimethamine the most common, but also clindamycin, azithromycin, clarithromycin, dapsone and atavaquone) must sometimes be prescribed indefinitely since, although they are lethal for the parasite *in vitro*, these substances do not always eliminate the parasite from the body. Since these treatments are sometimes incompatible with tritherapy, prophylaxis is difficult. In the battle which is still to be fought against AIDS, it is therefore fundamental to investigate novel treatments capable of eradicating *Toxoplasma*.

Other apicomplexes, such as those of the *Eimeria* genus, are responsible for coccidiosis in birds and cattle.

Recently, it has become known that these parasites have plant subcellular structures (McFadden et al., *Nature*, 1996, **384**, 482; Köhler et al., Science, 1997, **275**, 1485-1489), termed apicoplasts.

These authors have identified, by *in-situ* hybridization, the plast which contains a 35 kb DNA in *Toxoplasma gondii*: it is an organelle limited by 4 membranes, which is close in evolution to that of green algae. This plast has very rapidly been presented as a weakness of apicomplex parasites (Fichera et Roos,

Nature, 1996, **390**, 407-409). These authors have in particular shown that some antibiotics, such as fluoroquinolones and macrolides, inhibit prokaryotic DNA gyrases and block the replication of this 35 kb DNA, which appears to be necessary for the survival of the parasite. More recently, Waller et al. (PNAS, 1998, **95**, 12352-12357) have shown that this plast contains a protein known to synthesize fatty acids in plant chloroplasts, acyl carrier protein or ACP. The ACP precursor contains a transit sequence of the chloroplast type, which allows the protein (the mature ACP, or a fluorescent label of the GFP type fused with the transit sequence of the ACP precursor) to be integrated into the parasite plast.

ACP is not, unfortunately, specific for the plant kingdom, and is found in particular in bacteria of the intestinal tract. It does not therefore constitute a specific target for a medicinal product which would affect only the apicomplexes living as parasites in the body.

The aim of the inventors has therefore been to provide a target specific for apicomplex parasites, in order to select novel medicinal products which are effective against said apicomplex parasites.

They have now found that MGDG synthase (an enzyme which is essential for the biogenesis of the plast envelope) may be a target of choice for active principles against *Plasmodium* (malaria), *Toxoplasma* (toxoplasmosis) and *Eimeria* (coccidiosis), and for herbicides.

Specifically, MGDG (monogalactosyldiacylglycerol, figure 1) is known to be in all the plasts analyzed to date: it is the most abundant lipid of plastidial membranes (> 50% of the glycerolipids), is vital to plast biogenesis and cell survival and does not exist in the other membrane systems, in particular in animal

cells (Douce, Science, 1974, **183**, 852-853); the biosynthesis thereof is catalyzed in the envelope by a UDP-galactose: 1,2-diacylglycerol 3-ß-D-galactosyl-transferase (EC 2.4..1.46), also named MGDG synthase, according to the following reaction:
1,2-diacylglycerol + UDP-galactose → UDP + 1,2-diacyl-3-O-ß-D-galactopyranosyl-*sn*-glycerol.

A subject of the present invention is the use of an MGDG synthase for selecting or screening products which inhibit the activity of MGDG synthase and which can be used as active principles against apicomplex parasites, and in particular those responsible for malaria, for toxoplasmosis and for coccidiosis.

A subject of the present invention is also the use of a plastidial membrane isolated from a plant, for selecting or screening products which inhibit the activity of MGDG synthase and which can be used as active principles against apicomplex parasites, and in particular those responsible for malaria, for toxoplasmosis and for coccidiosis.

A subject of the present invention is also the use of an MGDG synthase for selecting or screening products which inhibit the activity of MGDG synthase and which can be used as herbicides.

A subject of the present invention is also the use of a plastidial membrane isolated from a plant, for selecting or screening products which inhibit the activity of MGDG synthase and which can be used as herbicides.

A subject of the present invention is also a method for screening and for selecting apicomplex antiparasitic agents and/or herbicides, characterized in that it comprises:

- incubating a substance to be tested with an MGDG synthase and

- measuring the specific enzymatic activity, after said incubation.

Inhibition of the enzymatic activity is defined by a decrease in the activity of at least 50%, as a percentage for control activity (activity of the enzyme treated as the test, but in the absence of inhibitor).

In accordance with the invention, said MGDG synthase preferably has an initial specific activity of between 0.1 and 120 µmol of galactose incorporated/h/mg of protein; some recombinant MGDG synthases may have a specific activity greater than 120 µmol of galactose incorporated/h/mg of protein.

In addition, in accordance with the invention, the MGDG synthase is of plant origin (spinach, cucumber or Arabidopsis, in particular) and is selected from the group consisting of the purified or recombinant MGDG synthases A and MGDG synthases B.

In accordance with said method, the MGDG synthase/substance to be tested incubation is carried out in an incubation medium containing a buffer adjusted to a pH of between 6 and 9 (MOPS-NaOH, Tris-HCl, KH_2PO_4/K_2HPO_4, 10 to 250 mM CAPS), in the presence of detergents (3 to 6 Mm CHAPS, or LDAO) of a reducing agent (1-10 mM DTT, or β-mercaptoethanol), of phosphatidylglycerol (0.1-2 mM) and of a salt (KCl or NaCl, 10-300 mM); preferably, said buffer contains 50 mM of MOPS-NaOH, pH 7.8, 4.5 mM of CHAPS, 1.3 mM of phosphatidylglycerol, 1 mM of DTT, 250 mM of KH_2PO_4/K_2HPO_4 and 250 mM of KCl.

Also in accordance with the invention, the enzymatic activity of the MGDG synthase is measured after

constituting micelles, in accordance with the method described in Maréchel et al. (*J. Biol. Chem.*, 1994, **269**, 5788-5798).

In accordance with the invention, said apicomplex parasite is selected from the group consisting of *Plasmodium, Toxoplasma* and *Eimeria*.

A subject of the present invention is also the use of an MGDG synthase inhibitor selected in accordance with the method defined above, for producing a medicinal product against parasites.

A subject of the present invention is also the use of an MGDG synthase inhibitor selected in accordance with the method defined above, as a herbicide.

Besides the arrangements above, the invention also comprises other arrangements which will emerge from the following description, which refers to examples of implementation of the method which is the subject of the present invention and also to the attached diagrams, in which:

- figure 1 represents MGDG (monogalactosyldiacylglycerol);

- figure 2 is a comparison of spinach, cucumber and Arabidopsis MGDG synthase; figure 2A corresponds to a comparison of the amino acid sequences deduced from the cDNAs encoding the various MGDG synthases; in this figure, atMGD A and atMGD B correspond to sequences derived from *Arabidopsis thaliana*, csMGD A corresponds to a sequence derived from *Cucumis sativa* and soMGD A corresponds to a sequence derived from *Spinacia oleracea*. * and : represent symbols for the identical amino acids and the conserved substitutions, respectively; h1 to h7 correspond to 7 putative

α-helices; figure 2B represents a phylogenic tree of mature MGDG synthases;

- figure 3 corresponds to the identification of the *r*MGD A reaction product; figure 3A: separation of the polar lipids by two-dimensional thin layer chromatography; in this figure, MGDG = monogalactosyl-diacylglycerol; DGDG = digalactosyldiacylglycerol; TGDG = trigalactosyldiacylglycerol; SL = sulfolipid; PC = phosphatidylcholine; PG = phosphatidylglycerol; figure 3B corresponds to the analysis of the galactolipids synthesized *in vitro* by the *r*MGD A;

- figure 4 illustrates the location of the *r*MGD A in *E. coli*;

- figure 5 corresponds to the partial purification of the *r*MGD A; figure 5A: fractionation by hydroxyapatite agarose chromatography; figure 5B: SDS-Page analysis of the fraction eluted from the hydroxyapatite agarose column.

It should be clearly understood, however, that these examples are given by way of illustration of the subject of the invention, of which they in no way constitute a limitation.

Example 1: Preparation of an MGDG synthase, from a plant

The MGDG synthase is solubilized and purified from the envelope of spinach chloroplasts, under the conditions set out in Maréchal et al. (C.R. Acad. Sci. Paris, 1991, **313**, III, 521-528; *J. Biol. Chem.*, 1994, **269**, 8, 5788-5798; *J. Biol. Chem.*, 1995, **270**, 11, 5714-5722).

Specifically:

- the envelope membranes of spinach chloroplasts are purified (see Maréchal et al., *J. Biol. Chem.*, 1995, mentioned above).

The envelope membranes may also be obtained in accordance with the following technique: more precisely, all procedures are carried out at 0°-5°C. The chloroplasts are obtained from 3-4 kg of spinach leaves (*Spinacia oleracea* L.) and purified by isopycnic centrifugation using Percoll gradients. The purified intact chloroplasts are lyzed in a hypotonic medium and the envelope membranes are purified from the lysate by centrifugation in a sucrose gradient.

The envelope membranes obtained are stored under liquid nitrogen, in the medium comprising 50 mM of MOPS-NaOH, pH 7.8 and 1 mM of DTT (dithiothreitol);

- the MGDG synthase is solubilized and purified from the envelope membranes obtained, as specified above (see Maréchal et al., *J. Biol. Chem.* 1995, mentioned above).

The MGDG synthase can also be obtained from cucumber (application JP 10014579 in the name of Kirin Brewery Co. Ltd).

In the context of the implementation of the method according to the present invention, it is preferable to use an MGDG synthase having at least a specific activity of 0.1 µmol of galactose incorporated/h/mg of protein.

Example 2: Preparation of a recombinant MGDG synthase and constructs for the overexpression thereof in *E. coli*.

1) Cloning and overexpression of a class A MGDG [lacuna] in *E. coli*.

- cloning of the MGDG synthase cDNA:

A 1647 bp fragment corresponding to the mature protein of the cucumber MGDG [lacuna] cDNA (Shimojima et al., 1997) is used as a probe to screen a λgt11 library obtained from spinach leaves.

Before screening, the presence of a homologous mRNA is verified by *Northern blot* on total RNA from spinach leaves. 320,000 plaques are cultured on *E. coli* Y1090 and transferred onto Hybond-N^+ membranes. The membranes are prehybridized for 2 h at 60°C in a solution comprising 2 × SSC, 5 × Denhardt's, 0.5% SDS (w/v) and salmon sperm DNA (0.1 mg/ml^{-1}).

The hybridization is carried out for 16 h at 60°C in the same reagent in the presence of 1 ng of [α-^{32}P]dCTP-labeled cucumber DNA. The membranes are washed 3 times for 3 min at room temperature in 2 × SSC, 0.1% SDS (w/v) and twice for 15 minutes at 55°C, and then autoradiographed.

Two positive clones are then purified by 3 rounds of screening. The phage DNA is extracted and digested with *EcoRI* or *EcoRI* and *BamHI*. The two cDNA inserts are subcloned into the pBlueScript SK+ plasmid (digested with *EcoRI* or digested with *EcoRI-BamHI*), for sequencing.

The analysis of the restriction fragment and the sequencing show that the two clones correspond to the same cDNA.

The PCR amplification with primers adjacent to the λgt11 cloning site reveals inserts of 2.5 and 0.9 kb, respectively. The analysis of the sequence of the inserts obtained by PCR shows that the 0.9 kb insert is identical to the 3′ end of the 2.5 kb insert.

Consequently, the longest insert is cleaved with the *BamHI/EcoRI* restriction enzymes, subcloned into the pBlueScript SK+ plasmid (stratagene) and sequenced.

The sequence obtained, which comprises 1851 nucleotides, appears to be a chimera. It contains an 807 nucleotide sequence which is highly homologous to the coding end in 3' of the cucumber MGDG synthase cDNA, including the stop codon. This truncated DNA is fused at its 5′ end with a partial DNA sequence (1044 nucleotides) homologous to β-endoglucanases. The 5′ end of the MGDG synthase cDNA is cloned by rapid amplification of cDNA ends (RACE) using the Marathon amplification kit (Clontech).

The spinach leaf cDNA is prepared from polyA+ mRNA and used as a matrix for the PCR amplifications of the 5′ end of the MGDG synthase cDNA, in accordance with the manufacturer's instructions. The specificity of the reaction comes from the specific primer CTCATTTGAAGGGCAGTAGCACC (nucleotides 870 to 848) (SEQ ID No. 1) and through "hot start" PCR.

This method makes it possible to clone a 1001 bp fragment which is then subcloned into the pBlueScript SK+ plasmid and sequenced on both strands in 3 independent clones, so as to be sure that the Taq polymerase does not introduce any error.

The 5′ RACE fragment includes an identifiable initiation codon and 131 nucleotides of the 5′ untranslated sequence. The clone comprising the complete MGDG synthase cDNA is generated from the

spinach leaf cDNA by PCR, using primers specific for the 3' and 5' ends of the cDNA. The sense primer is as follows: CACACAATATTTCCAATGTATACCCAC (nucleotides -82 to -57) (SEQ ID No. 2).

The antisense primer is as follows: GATTATCATTTCCCCTCGCCCTGCC (nucleotides 1672 to 1648) (SEQ ID No. 3).

The 1765 bp DNA fragment obtained is subcloned into the pBlueScript SK+ plasmid at the *SmaI* restriction site, and the sequence is verified.

The cDNA sequence is shorter than the transcript (2.5 kb) detected by *Northern blot* analysis, thus indicating that it is not complete.

The 2 techniques combined (5'-RACE technique and screening of a spinach cDNA library) make it possible to obtain a 1890 bp sequence which includes a 1569 bp open reading frame encoding a 522 amino acid protein (57.5 kDa) (figure 2) which belongs to the MGDG synthase A family.

The analysis of the amino acid sequence shows that this MGDG synthase A contains more nonpolar (56%) than polar (44%) residues, 9 cystein residues and 16 histidine residues which may be involved in the chelation of metals; this protein has a basic isoelectric point (pI = 9.16).

2) Extraction

.extraction of the recombinant MGDG synthase (*r*MGD A)

all the procedures are carried out at 4°C. A pellet of recombinant bacteria (34 mg of protein) expressing the MGDG synthase (7 mg of protein) is resuspended in 50 ml of medium A (6 mM of CHAPS, 50 mM of MOPS-NaOH, pH 7.8, 1 mM of DTT) containing 50 mM of KH_2PO_4/K_2HPO_4 and a

mixture of protease inhibitors (1 mM of PMSF; 1 mM of benzamidine; 0.5 mM of caproic acid). After cell lysis by repeated sonication, the suspension is mixed at 0°C in ice for 30 minutes. The mixture is centrifuged for 15 min at 243 000 g (Beckman L2, SW 40 rotor). The supernatant containing the solubilized proteins (16 mg) is loaded onto a hydroxyapatite ultrogel (IBF-France) column (Pharmacia C10/20, 25 ml of gel), equilibrated with a medium A containing 50 mM of KH_2PO_4/K_2HPO_4. The proteins are eluted using a gradient of KH_2PO_4/K_2HPO_4 (50-275 mM) (in a medium A; flow rate: 30 ml/h; fraction volume: 1.5 ml). The recombinant MGDG synthase is eluted at 275 mM of KH_2PO_4/K_2HPO_4.

3) Overexpression of the spinach MGDG synthase in *E. coli*

- Materials and methods

Two mature forms of MGDG synthase are overexpressed in *E. coli*, using the pET-15b plasmid (Novagen) and a plasmid, termed pET-Y 3a, which makes it possible to overcome the problem which comes from the fact that the deduced sequence of the MGDG synthase contains 22 arginine residues, 17 of which are encoded by AGG or AGA, these being codons which are, in fact, used very little in *E. coli*. Specifically, pET-Y 3a was constructed by inserting, into the pET-3a plasmid (Novagen), the arg U (or DNA Y) gene encoding the transfer RNA for arginine, associated with the rare AGA/AGG condons.

These two plasmids are linearized with *BamHI* and *NdeI*. The PCR-amplified fragments are generated from the complete cDNA clone. The pET-15 plasmid is ligated with a fragment encoding the 417 C-terminal residues of the enzyme, which is amplified by PCR using the following primers:

sense primer: GGAG*CATATG*GGGGTGAGTGATAATG (SEQ ID No. 4)

and
antisense primer: GTTCT*GGATCC*TCAAGCAGCACAAGAGT (SEQ ID No. 5)
and digested with the *BamHI* and *NdeI* enzymes.

Another fragment digested with the *BamHI* and *NdeI* enzymes, encoding the 424 C-terminal residues of the enzyme, is amplified by PCR using the following primers:
sense primer: CTTCA*CATATG*CTTAATTCCGGGGAGAG (SEQ ID No. 6) and
antisense primer: GTTCT*GGATC*TCAAGCAGCACCGAGTA (SEQ ID No. 7),
and is subcloned into the *BamHI-NdeI* restriction site of the pET-Y3 plasmid.

The first construct allows the expression of a histidine-tagged fusion protein (*h*MGD A) comprising 437 residues (48.24 kDA). The second construct allows the expression of a 425 amino acid protein (*r*MGD A) including an additional initiation methionine corresponding to the ATG codon of the *BamHI* restriction site. The recombinant proteins are expressed in *E. coli* BI.21(DE3). The bacterial cultures are cultured at 37°C, with vigorous shaking (Certomat, 250 rpm), until an optical density of 0.4 to 0.6 is obtained. Recombinant MGDG synthase expression is induced by adding 0.4 mM of IPTG to the medium and the cultures are incubated for 3 h at 25°C. The bacteria are pelleted by centrifugation (Eppendorf, 14 000 g, 10 min) and solubilized in a buffer A (50 mM MOPS, pH 7.8, 10 mM DTT, 1 mM EDTA, 1 mM benzamidine, 1 mM PMSF and 0.5 mM caproic acid) in the presence or absence of 0.1% Triton X-100 or in a buffer A with 6M urea. The soluble and insoluble fractions are separated by centrifugation (Airfuge, 115 000 g, 15 min) and analyzed on SDS-PAGE (12% polyacrylamide gel). The proteins are detected by staining with Coomassie blue.

The *h*MGD is purified to homogeneity from the bacteria by affinity chromatography based on a metal (NTA, Novagen), followed by desalification through a PD10 column (Pharmacia) equilibrated in a mixture comprising 5 mM imidazole, 0.5 mM NaCl and 20 mM Tris-HCl, pH7.9, in the presence of 6M urea.

The pure recombinant protein (1 mg) is used to obtain a rabbit polyclonal antibody (Eurogentec, Belgium). The IgG is purified by DEA-trisacryl M (IBF, France) chromatography.

- Results

In order to minimize the effect of the N-terminal end of the target chloroplast sequence, the spinach cDNA is expressed from the residue leucine 99, which corresponds to the putative cleavage site of the signal peptide of the cucumber MGD A precursor (Shimojima et al., PNAS, 1997, **94**, 333-337).

Using UDP-[^{14}C]gal as substrate, it is possible to measure the MGDG synthase activity in the extracts of *E. coli* expressing the *r*MGD A, after induction with IPTG: more than 2 µmol of galactose are incorporated/h/mg of protein. The activity determined in the extracts of *E. coli* containing the histidine-tagged protein hMGD A is of the same order (1.3 µmol of galactose incorporated/h/mg of protein).

Only an insignificant fraction of [^{14}C]-galactose (less than 0.1 µmol of galactose incorporated/h/mg of protein) is observed in the *E. coli* lipids before induction with IPTG. In addition, no [^{14}C]-galactose incorporation is observed in the control bacteria, which express E37, another inner envelope protein (Teyssier et al., Plant J., 1996, **10**, 903-912). After 3 h of induction with IPTG, an extract of *E. coli* containing the overexpressed *r*MGD A is incubated in the

presence of UDP-[^{14}C]-gal, and the lipids are extracted in order to analyze the reaction products.

The lipid extract is analyzed by two-dimensional thin layer chromatography, at the same time as the envelope lipids added to the mixture as a standard (Douce et al., *In Methods in Plant Biochemistry, Lipids, Membranes and Aspects of Photobiology* (Harwood et al. eds, 1990, **4**, 71-103, Academic Press, London). Figure 3A shows that a single radioactive spot comigrates with the MGDG of origin and is detected by autoradiography. A more extensive characterization of the MGDG was carried out by analyzing the polar groups by two-dimensional paper chromatography; in this case also, a single radioactive spot is detected by autoradiography and comigrates with the glyceryl galactose obtained after deacylation of the envelope MGDG by gentle alkali hydrolysis (figure 3B).

These results show that the product formed in *E. coli* is effectively MGDG, which is normally absent in *E. coli* membranes. No other lipid containing galactose is formed, unlike that which is observed after incubating isolated envelope membranes in the presence of UDP-[^{14}C]-gal.

MGDG synthase activity is catalyzed by a multigenic family of proteins.

The bireactional mechanism of MGDG synthase activity has been studied using very enriched membrane protein fractions, as has its selectivity for various molecular species of 1,2-diacylglycerol (Maréchal et al., J. Biol. Chem., 1994, **269**, 5788-5798). Certain structural properties of the catalytic site have been elucidated: the existence of amino acids which are important for the catalysis (Cys, His, Lys) and the association of the enzyme with divalent metals (Maréchal et al., J. Biol. Chem., 1995, 270, 5714-5722). The functional

molecular mass during the inactivation of MGDG synthase has, moreover, been determined by gamma irradiation: the apparent molecular mass of envelope MGDG synthase is 97 ± 5 kDa. Since the mature MGDG synthase polypeptide is close to 45 kDa in size in a denaturing gel, it is probable that, in the envelope, the MGDG synthase is in dimeric form. A functional molecular mass of 114 ± 12 kDa has also been deduced, using the same technique, for purified recombinant MGDG synthase A. This result suggests that MGDG synthases are probably homodimers.

Example 3: Measurement of the enzymatic activity, using micelles

The activity of the MGDG synthase is measured on various types of sample, depending on the model chosen: plastidial membrane, membrane fractions of *E. coli* overexpressing a recombinant MGDG synthase (rMGD A, 0.7 µg protein/assay, enzyme extracted beforehand from a plant (see Example 1)).

. preparation of micelles

1.3 mM of phosphatidylglycerol (PG) and 160 µM of diacylglycerol (DAG) are dissolved in chloroform. After evaporating the solvent under argon, 200 µl of incubation medium containing 50 mM of MOPS-NaOH, pH 7.8, 4.5 mM OF CHAPS, 1 mM of DTT, 250 mM of KH_2PO_4/K_2HPO_4 and 250 mM of KCl are added and the medium is mixed vigorously so as to resuspend the lipids. 100 µl of fractions containing the MGDG synthase, in the incubation medium, are introduced then the medium is again mixed vigorously and then maintained at 20°C for 1 h.

This procedure makes it possible to obtain micelles, in accordance with Maréchal et al., 1994, mentioned above.

.enzymatic reaction

The reaction in the incubation mixture is then initiated by adding 1 mM of UDP-[^{14}C]gal (37 Bq/µmol). After 10 min to 1 h, the reaction is stopped by adding a chloroform/methanol mixture (1:2, v/v), the lipids are extracted in accordance with the method of Bligh et al (Can. J. Biochem. Physiol, 1959, **37**, 911-917) and the radioactivity of the labeled galactolipids is determined by liquid scintillation counting as described in Covès et al. (FEBS lett., 1986, 208, 401-406). The activity is expressed in µmol of galactose incorporated/h/mg of protein.

A high specific activity, i.e. up to 115-120 µmol of galactose incorporated/h/mg of protein, and even more, can be obtained in a sample rich in MGDG synthase.

3) Specific activity of the overexpressed soluble rMGD A

When the expression of the spinach MGDG synthase in *E. coli* is analyzed, it is observed that most of the protein (rMGD A) is insoluble and that detergents (6 mM of CHAPS or 1% of Triton X-100) only partially solubilize the protein (figure 4). On the other hand, almost all the overexpressed protein is solubilized by urea, indicating that most of the MGDG synthase is present in inclusion bodies (figure 4). In this fraction, the activity of the MGDG synthase is very low (0.03 µmol of galactose incorporated/h/mg of protein). In fact, the hydroxyapatite chromatography analysis of the *E. coli* fractions solubilized by CHAPS shows that only a small fraction (approximately 0.1%) of the recombinant protein synthesized by the bacterium is active. The experimental conditions used are the same as those used for the envelope MGDG synthase (see above).

In figure 4, the *r*MGD A expression is induced with 0.4 mM of IPTG as specified in Materials and methods above.

Most (50 to 80%) of the activity loaded at the top of the column is found in a narrow peak which is eluted with 275 mM phosphate (figure 5A). In this peak, the specific activity of the MGDG synthase is very high: 115 µmol of galactose incorporated/h/mg of protein.

The analysis of the polypeptides present in the various fractions shows that a 45 kDa polypeptide corresponding to the *r*MGD A is present in the active fractions, but also in the dead volume, in which most of the protein is present in an inactive form (figure 5B). This shows that only a fraction (1%) of the protein solubilized by CHAPS is effectively active.

In this figure 5B, 20 µl of fraction eluted from the hydroxyapatite column are analyzed by SDS-PAGE (12% polyacrylamide gel); the proteins are detected by staining with Coomassie blue; Lo: sample loaded at the top of the column; 15, 30, etc.: fractions eluted from a column; MW: molecular weight marker (Biorad); the *r*MGD A is indicated with an arrow and the active *r*MGD A is found only in fraction 67 to 71.

Example 4: Comparison of the biochemical properties of the overexpressed MGD A, with the chloroplast envelope MGDG synthase

The analysis of the activity of the MGDG synthase partially purified from spinach leaf chloroplasts (Maréchal et al., J. Biol. Chem., 1995, **270**, 5714-5722) demonstrated that DTT can protect the activity of the enzyme against oxidation and that N-ethylmaleimide (NEM) and orthophenanthroline are powerful inhibitors of the enzyme.

The overexpressed spinach MGDG synthase has the same properties.

The *r*MGD A, purified by hydroxyapatite chromatography, is very active in the presence of DTT. If the DTT is removed by chromatography on a Biogel P6-DG column, the MGDG synthase loses 85% of its activity, whereas the addition of DTT maintains its activity.

The fractions of partially purified *r*MGD A are desalified by chromatography on a Biogel P6-DG (Bio-Rad) column (Pharmacia, C10/40 column, 30 ml of gel) equilibrated in DTT. Aliquots (200 µl) of the fractions are incubated for 40 minutes at 25°C with gentle stirring, in the presence or absence of DTT. The galactosylation activity is then measured as specified above (see Example 3).

The results obtained are summarized in Table I below:

Table I

Fraction purified on hydroxyapatite	Activity (%)
Not desalified	100
Desalified	15
Desalified + 1 mM DTT	75
Desalified + 10 mM DTT	65

The activity is expressed as a percentage of the control (not desalified) activity.

Table II shows that the *r*MGD A is very sensitive to NEM and that protection of the activity is obtained by preincubation in the presence of DAG and/or of PG.

Table II

Pre-incubation (30 min)	Incubation +/- 150 µM NEM (10 min)	Incubation +/- 10 mM DTT (10 min)	Enzymatic reaction	Activity (%)
DTT	-	-	+PG+DAG+UDP-gal	100
-	-	+	+PG+DAG+UDP-gal	101
-	-	-	+PG+DAG+UDP-gal	37
-	+	+	+PG+DAG+UDP-gal	35
DTT	+	-	+PG+DAG+UDP-gal	108
UDP-gal	+	+	+PG+DAG	32
PG	+	+	+DAG+UDP-gal	56
PG+DAG	+	+	+UDP-gal	60

In order to obtain the results summarized in Table II, the *r*MGD A is desalified by chromatography on a Biogel P6-DG (Bio-Rad) column (Pharmacia, C10/40, column, 30 ml of gel) equilibrated in DTT. Aliquots (200 µl) of the fractions are incubated for 30 minutes at 25°C with gentle stirring, followed by a 10 min incubation in the presence or absence of 150 µM NEM and then a 10 min incubation in the presence or absence of DTT. The galactosilyation activity is then measured as specified above (see Example 3).

The activity is expressed as a percentage of the control activity, i.e. after incubation for 50 min in the presence of 10 mM DTT.

It is also observed that the overexpressed *r*MGD is inhibited by the hydrophobic chelating agent orthophenanthroline, as shown in Table III below:

Table III

Conditions	Other additions	Activity (%)
Initial activity (time 0)	-	100
Without orthophenanthroline	-	82
	PG	87
	PG + DAG	78
	UDP-gal	72
With orthophenanthroline	-	43
	PG	27
	PG + DAG	92
	UDP-gal	17

The inactivation of the *r*MGD A by orthophenanthroline is blocked by DAG, but is not affected by UDP-gal.

As emerges from the above, the invention is in no way limited to its methods of implementation, preparation and application which have just been described more explicitly; on the contrary, it encompasses all the variants thereof which may occur to a person skilled in the art, without departing from the context or from the scope of the present invention.

CLAIMS

1. The use of an inhibitor of the activity of MGDG synthase, for preparing a medicinal product against apicomplex parasites.

2. The use as claimed in claim 1, characterized in that said apicomplex parasite is selected from the group consisting of *Plasmodium*, *Toxoplasma* and *Eimeria*.

3. The use of an inhibitor of the activity of MGDG synthase, as a herbicide.

4. A method for screening and for selecting antiparasitic agents and/or herbicides, characterized in that it comprises:

- incubating a substance to be tested with an MGDG synthase or with a plastidial membrane isolated from a plant and

- measuring the specific enzymatic activity, after said incubation.

5. The method as claimed in claim 4, characterized in that said MGDG synthase preferably has an initial specific activity of between 0.1 and 120 µmol of galactose incorporated/h/mg of protein.

6. The method as claimed in claim 4 or claim 5, characterized in that the MGDG synthase/substance to be tested incubation is carried out in an incubation medium containing a buffer adjusted to a pH of between 6 and 9, in the presence of detergents, of a reducing agent, of phosphatidylglycerol and of a salt.

7. The method as claimed in claim 6, characterized in that the incubation medium preferably contains 50 mM of MOPS-NaOH, pH 7.8, 4.5 mM of CHAPS, 1 mM of DTT, 1.3 mM of phosphatidylglycerol, 250 mM of KH_2PO_4/K_2HPO_4 and 250 mM of KCl.

8. The method according to any one of claims 4 to 7, characterized in that the MGDG synthase is of plant origin and is selected from the group consisting of the purified or recombinant MGDG synthases A and MGDG synthases B.

9. The method as claimed in any one of claims 4 to 8, characterized in that said apicomplex parasite is selected from the group consisting of *Plasmodium*, *Toxoplasma* and *Eimeria*.

10. The use of an MGDG synthase inhibitor selected in accordance with the method as claimed in any one of claims 4 to 8, for producing a medicinal product against apicomplex parasites.

11. The use of an MGDG synthase inhibitor selected in accordance with the method as claimed in any one of claims 4 to 8, as a herbicide.

Abstract

The invention concerns a method for screening and selecting parasiticides (apicomplex phyllum parasites) and/or herbicides and the uses thereof. Said method consists in incubating a substance to be tested with a MGDG synthase and measuring the specific enzymatic activity, after said incubation. The invention also concerns the use of MGDG synthase or a plant isolated plastid membrane for selecting or screening the products inhibiting the activity of the MGDG synthase, capable of being used as active principles against apicomplex parasites and/or of being used as herbicides.

09/926169

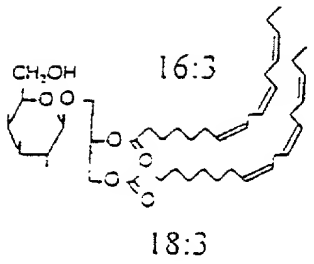

FIGURE 1

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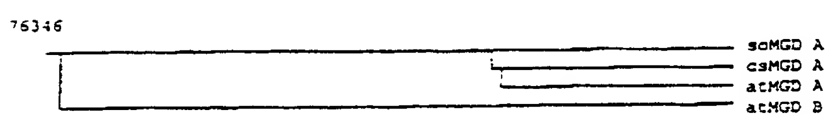

FIGURE 2

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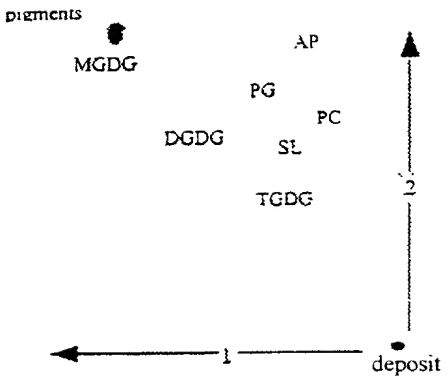

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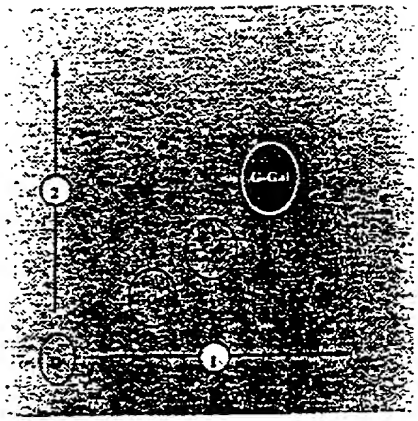

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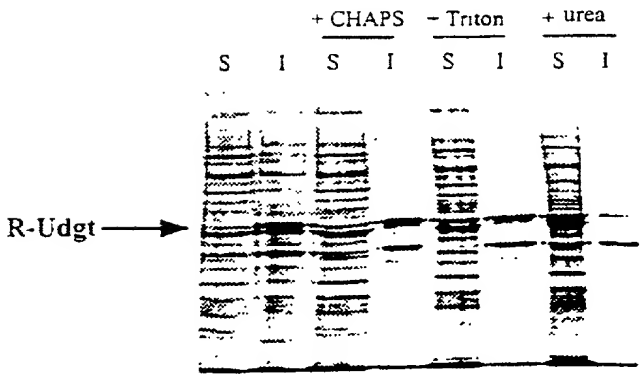

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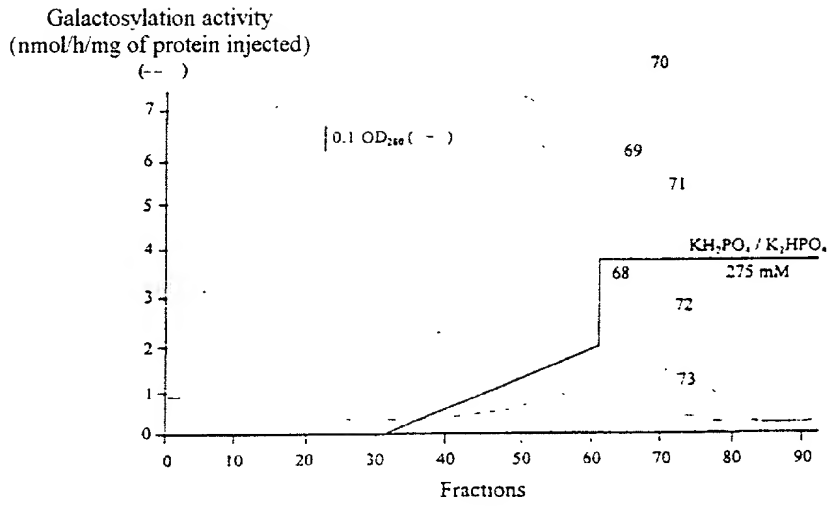

FIGURE 5A

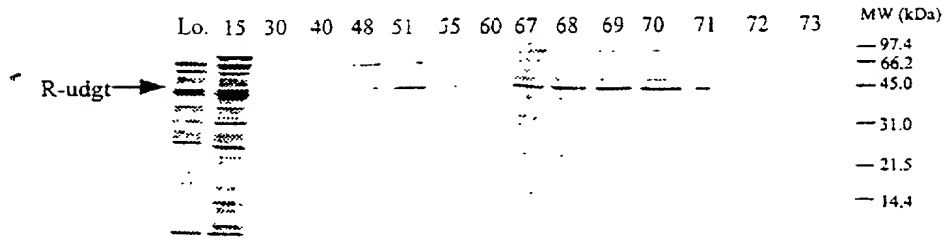

FIGURE 5B

#4

Declaration and Power of Attorney for Patent Application
Déclaration et Pouvoirs pour Demande de Brevet
French Language Declaration

En tant l'inventeur nommé ci-après, je déclare par le présent acte que :

As a below named inventor, I hereby declare that :

Mon domicile, mon adresse postale et ma nationalité sont ceux figurant ci-dessous à côté de mon nom.

My residence, post office address and citizenship are as stated next to my name.

Je crois être le premier inventeur original et unique (si un seul nom est mentionné ci-dessous), ou l'un des premiers co-inventeurs originaux (si plusieurs noms sont mentionnés ci-dessous) de l'objet revendiqué, pour lequel une demande de brevet a été déposée concernant l'invention intitulée

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed an for which a patent is sought on the invention entitled

SCREENING METHOD INVOLVING MGDG SYNTHASE

et dont la description est fournie ci-joint à moins

the specification of which :

☐ ci-joint

☐ is attached hereto.

☐ a été déposée le

☒ was filed on

sous le numéro de demande des Etats-Unis ou le numéro de demande international PCT

as United States Application Number or PCT International Application Number.
PCT/FR00/00658 filed on March 17, 2000

et modifiée le

and was amended on

(le cas échéant).

(if applicable).

Je déclare par le présent acte avoir passé en revue et compris le contenu de la description ci-dessus, revendications comprises, telles que modifiées par toute modification dont il aura été fait références ci-dessus.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

Je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations,§ 1.56.

French Language Declaration

Je revendique par le présent acte avoir la priorité étrangère, en vertu du Titre 35, § 119(a)-(d) ou § 365(b) du Code des Etats-Unis, sur toute demande étrangère de brevet ou certificat d'inventeur ou, en vertu du Titre 35, § 365(a) du même Code, sur toute demande internationale PCT désignant au moins un pays autre que les Etats-Unis et figurant ci-dessous et, en cochant la case, j'ai aussi indiqué ci-dessous toute demande étrangère de brevet, tout certificat d'inventeur ou toute demande internationale PCT ayant date de dépôt précédant celle de la demande à propos de laquelle une priorité est revendiquée.

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below, and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign application(s)
Demande(s) de brevet antérieure(s) dans un autre pays.

(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Anné de dépôt)	Priority claimed Droit de priorité revendiqué ☒ Yes / Oui	☐ No / Non
99/03434	**FRANCE**	**19/03/1999**		
(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Anné de dépôt)	☐ Yes / Oui	☐ No / Non

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 119(e) du Code des Etats-Unis, de toute demande de brevet provisoire effectuée aux Etats-Unis et figurant ci-dessous.

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 120 du Code des Etats-Unis, de toute demande de brevet effectuée aux Etats-Unis, ou en vertu du Titre 35, § 365© du même Code, de toute demande internationale PCT désignant les Etats-Unis et figurant ci-dessous et, dans la mesure où l'objet de chacune des revendications de cette demande de brevet n'est pas divulgué dans la demande antérieure américaine ou internationale PCT, en vertu des dispositions du premier paragraphe du Titre 35, § 112 du code des Etats-Unis, je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations, dont j'ai pu disposer entre la date de dépôt de la demande antérieure et la date de dépôt de la demande nationale ou internationale PCT de la présente demande :

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)	(Status) (patented, pending,abandoned) (Statut) (breveté, en cours d'examen, abandonné)
(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)	(Status) (patented, pending,abandoned) (Statut) (breveté, en cours d'examen, abandonné)

Je déclare que par le présent acte que toute déclaration ci-incluse est, à ma connaissance, véridique et que toute déclaration formulée à partir de renseignements ou de suppositions est tenue pour véridique ;et de plus, que toutes ces déclarations ont été formulées en sachant que toute fausse déclaration volontaire ou son équivalent est passible d'une amende ou d'une incarcération, ou des deux, en vertu de la section 1001 du Titre 18 du Code de Etats-Unis, et que de telles déclarations volontairement fausses risquent de compromettre la validité de la demande de brevet ou du brevet délivré à partir de celle-ci.

I hebery declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true ; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

French Language Declaration

POUVOIRS : En tant que l'inventeur cité, je désigne par la présente l'(les) avocats(s) et/ou agent(s) suivant(s) pour qu'ils poursuive(nt) la procédure de cette demande de brevet et traite(nt) toute affaire s'y rapportant avec l'Office des brevets et des marquees : (mentionner le nom et le numéro d'enregistrement).

POWER OF ATTORNEY : As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to persecute this application and transact all bussiness in the Patent and Trademark Office connected therewith : (list name and registration number)

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(nom et numéro de téléphone)

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(703) 413-3000

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Nationalité Française	Citizenship
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(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

(Supply similar information and signature for third and subsequent joint inventors.)

French Language Declaration

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Nationalité Française	Citizenship
Adresse Postale 5, Rue du Palenka 38000 GRENOBLE (France)	Post Office Address
Nom complete du cinquième co-inventeur, le cas echeant Signature de l'inventeur Date	Full name of fifth joint inventor, if any Fifth inventor's signature Date
Domicile	Residence
Nationalité	Citizenship
Adresse Postale	Post Office Address
Nom complete du sixième co-inventeur, le cas echeant Signature de l'inventeur Date	Full name of sixth joint inventor, if any Sixth inventor's signature Date
Domicile	Residence
Nationalité	Citizenship
Adresse Postale	Post Office Address

(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

(Supply similar information and signature for third and subsequent joint inventors.)

SEQUENCE LISTING

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[ATOMIC ENERGY COMMISSION]
MARECHAL Eric
BLOCK Maryse
JOYARD Jacques
DOUCE Roland

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